VE001 - Interaction between *Trypanosoma rangeli* and the Rhodnius prolixus salivary gland depends on the phosphotyrosine ecto-phosphatase activity of the parasite

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Trypanosoma rangeli is the trypanosomatid that colonizes the salivary gland of its insect vector. In this study we investigated the role of the phosphotyrosine (P-Tyr) ecto-phosphatase activity of *T. rangeli* in its interaction with *Rhodnius prolixus* salivary glands. Long but not short epimastigotes adhered to the gland cells and the strength of interaction correlated with the enzyme activity levels in different strains. Differential interference contrast microscopy demonstrated that clusters of parasites are formed in most cases, suggesting cooperative interaction in the adhesion process. The tightness of the correlation was evidenced by modulating the P-Tyr ecto-phosphatase activity with various concentrations of inhibitors. Sodium orthovanadate, ammonium molybdate and zinc chloride decreased the interaction between *T. rangeli* and *R. prolixus* salivary glands in parallel. Levamisole, an inhibitor of alkaline phosphatases, affected neither process. When the P-Tyr ecto-phosphatase of living *T. rangeli* epimastigotes was irreversibly inactivated with sodium orthovanadate and the parasite cells were then injected into the insect thorax, colonization of the salivary glands was greatly depressed for several days after blood feeding. Addition of P-Tyr ecto-phosphatase substrates such as p-nitrophenyl phosphate (*p*NPP) and P-Tyr inhibited the adhesion of *T. rangeli* to salivary glands, but P-Ser, P-Thr and β-glycerophosphate were completely ineffective. Immunoassays using anti-P-Tyr-residues revealed a large number of P-Tyr-proteins in extracts of *R. prolixus* salivary glands, which could be potentially targeted by *T. rangeli* during adhesion. These results indicate that dephosphorylation of structural P-Tyr residues on the gland cell surfaces, mediated by a P-Tyr ecto-phosphatase of the parasite, is a key event in the interaction between *T. rangeli* and *R. prolixus* salivary glands. **Supported by:**:CNPq, CAPES, FAPERJ, FAPESP

VE002 - Lutzomyia longipalpis (Diptera; Psychodidae) saliva inhibits the classical pathway of Guinea pig (Cavia porcellus) and rat (Rattus norvegicus) complement system

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In the New World, females Lutzomyia longipalpis (Diptera; Psychodidae) are the main vectors of *Leishmania* infantum parasites, the causative agents of american visceral *Leishmania*sis (AVL). During blood feeding, the vector inoculates saliva into the host skin facilitating ingestion of blood since it contains a repertoire of pharmacologically active molecules, including immunomodulatory components. L. longipalpis saliva is able to inhibit human and canine complement system, a major mediator of vertebrates innate immune response and this inhibition may be involved in the parasite transmission and in the protection of vector midgut. Considering that AVL is a zoonosis and that L. longipalpis feeds on several animal species, it´s important to understand how the vector interacts with other host species. For checking if L. longipalpis saliva was able to inhibit Guinea pig and rat classical pathway, hemolytic assays were done by incubating sera of these animals at 37ºC with sheep sensitized red blood cells and different amounts of saliva. ELISA assays using IgG antibody-coated plates were also done for detecting C3b deposition in the presence or absence of the vector saliva. The action of the saliva on the alternative pathway was analyzed through hemolytic assays with rabbit red blood cells and ELISA assays with agarose-coated plates. Both assays for the two complement pathways were done at pH 7,4 and at pH 8,15 (the insect midgut pH right after blood meal). The results showed a dose-dependent inhibition of Guinea pig classical pathway in both pHs, but no effect was observed over the alternative pathway. Results of hemolytic assays with rat sera showed a similar effect over the classical pathway, but curiously, no inhibition of C3b deposition was observed. Thus, we may conclude that L. longipalpis saliva is able to inhibit the classical pathway of rodents complement system, probably acting in different points of the complement cascade. **Supported by:**:CNPq, FAPEMIG

VE003 - Effect of the parasites *Trypanosoma cruzi* and *Trypanosoma rangeli* in Rhodnius prolixus fitness

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Parasites can affect the fitness of their vertebrate hosts, as well as that of their insect vectors. This work investigated whether *Trypanosoma cruzi* and *Trypanosoma rangeli* affect aspects of the fitness of *Rhodnius prolixus*. We evaluated the effect of infection by *T. cruzi* on fertility/fecundity of *R. prolixus* adults reared at 25 and 30°C and the effect of infection by *T.rangeli* in adults reared at 26°C. The insects were infected with *T.cruzi* by offering them a blood-meal in an artificial feeder at 2nd instar. For infection with *T.rangeli*, an infective blood-meal was offered at 3rd instar and an inoculum with parasite loaded PBS at 4th instar. Control insects were only fed blood and later inoculated with PBS. Individual insects were weighed before and after blood feed as adults. For each treatment, 20 infected and 20 control couples were formed. The parameters were evaluated along 3 feeding/oviposition cycles (21 days each) for all 3 treatments. Females infected with *T.rangeli* laid fewer eggs (19.1+1.8 vs. 37.9+2.1) and the infection also decreased the egg hatching rate (56.6+4.8 vs. 74.9 +3.3%). The E-value (number of eggs/female weight x blood ingested x 1000) was lower for infected bugs (1.7+0.2 vs. 2.5+0.2). The E-value is proportional to insect reproductive efficiency at converting nutrients to eggs. For infection with *T. cruzi* tested at 25°C, females oviposited more than control ones (51.4+2.1 vs. 43.6+2.4), while hatching rate and E-value were not different between them. Nevertheless, *T.cruzi* infection induced a decrease in the number of eggs laid at 30ºC (38.9 +1.6 vs. 46.9+1.8) and in the hatching rate (77.5+3.6 vs. 87.8+2.2). The E-value was lower for infected insects (2.2+0.1), than for control ones (4.1+0.1). *T.rangeli*, and *T.cruzi* tested at 30°C, further affected the fitness of *R.prolixus*, while *T.cruzi* tested at 25°C appears to have had less relevant effects on the insects, suggesting that temperature could modulate triatomine-*T. cruzi* interaction. **Supported by:**:CPqRR/Fiocruz, Fapemig, INCT-EM

VE004 - PRELIMINARY CHARACTERIZATION OF THE foraging GENE OF Rhodnius prolixus: TRYPANOSOME INFECTION ALTERS GENE EXPRESSION AND LOCOMOTOR ACTIVITY PATTERNS

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The *foraging* gene (henceforth *for*) encodes a cGMP-dependent protein kinase (PKG) and has been related with a large array of insect behaviours, including food search, responses to stress, memory formation and learning. Behavioural changes due to the presence of pathogens have been reported in many insects. Recently, our group observed that trypanosome infected triatomines present altered locomotor activity patterns, shelter use and temperature preference. The objectives of the present study were to identify and characterize *Rhodnius prolixus for* gene at the molecular level. *For* gene sequences described for phylogenetically related insects were chosen as templates from genomic databases. These were compared with *R. prolixus* genome predicted proteins to obtain a candidate sequence. Wise2, BioEdit 7.5, MEGA5 and ClustalW2 softwares were used to evaluate this sequence and compare it with those of other insects. Identification was confirmed through bioinformatic analyses of sequences and domains. The IDT Primer Quest program was used to design specific primers for sequencing this gene. Alternative primers were produced for RT-PCR and qPCR studies in order to evaluate for expression profiles. Results showed that *for* gene is expressed at the central nervous system of bugs. Sequencing experiments confirmed the previous bioinformatic prediction. Finally, the levels of expression of *for* gene were compared by qPCR in healthy and *T. rangeli* infected insects. Infected insects showed gene *for* expression levels 2.6 times lower than healthy insects. Analysis of RNA expression in *Trypanosoma cruzi* infected insects is currently in progress. Understanding molecular mechanisms underlying triatomine behaviour and parasite induced alterations may allow uncovering new targets for bug control. **Supported by:**:CPqRR/FIOCRUZ, INCT-EM, FAPEMIG

VE005 - Vertebrate hosts are efficient reservoirs for the transmission of *Trypanosoma rangeli* to insect vector

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The development of *Trypanosoma rangeli* infection in mammal hosts is not well known, as replicative forms have never been described in circulating blood or inside cells. T. rangeli infection in mammals is characterized by a low number of circulating parasites which tend to disappear as infection progresses. The transmission of T. rangeli for R. prolixus during different mice infection stages was evaluated in this work. Swiss mice were individually exposed to the bite of a single infected 5th instar nymph. Simultaneously, a healthy 5th instar nymph was also allowed to feed in a way that no contact between the insects was possible. Later on, healthy 5th instar nymphs were individually fed on the mice infected before between days one and seven after their infection. This procedure was later repeated at days 15th and 30th post-infection, at which mice were also bled for hemoculture exams. Twenty days after being fed, insects had their intestinal contents and haemolymph examined for parasites. Of the twelve mice exposed to the bite of infected insects, 83% were infected according to xenodiagnosis and/or blood cultures. All nymphs that fed on mice simultaneously with infected ones presented infected intestines. Intestinal infection rates for insects fed on mice on subsequent days ranged between 80-90%, including those which fed on mice infected 15 or 30 days before. Nymphs who fed on 15/30 days infected mice presented higher numbers of parasites on their feces than those that did it in recently infected mice. Hemolymph infections were observed in 7% of the nymphs, regardless of the mice infection period at which they were infected. Our results probably reflect what happens in natural infections and show the high efficiency of T. rangeli transmission to vector insects. Evaluation of blood parasitemia in mice infected through a single bite is in progress to allow understanding how chronic mice infection produces larger amounts of parasites. **Supported by:**::CNPQ, FAPEMIG, CpQRR

VE006 - Partial transcriptome analysis of Triatoma brasiliensis anterior midgut using Expressed Sequence Tags (ESTs)

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Triatoma brasiliensis is a hematophagous arthropod and one of the main Brazilian vectors of *Trypanosoma cruzi*. *T. cruzi* host infection is modulated by the amount of blood ingested. So, the success in the feeding is directed related to the success of *T. cruzi* transmission. Due to this evident parasite-vector interaction, understanding what influences the insect feeding performance is of utmost importance. In this work, we propose the characterization of the anterior midgut transcriptome of *T. brasiliensis* by Expressed Sequence Tags (ESTs) analysis. Messenger RNA was isolated from the anterior midgut of fifth-instar nymphs and a cDNA library was constructed using the Creator™ Smart™ cDNA Library Construction Kit (Clontech). cDNA molecules were produced, amplified, size-selected and cloned into the pDNR-LIB vector. A total of 768 cDNA clones were randomly selected and sequenced on the MegaBace™ 1000 DNA sequencer (GE Healthcare) with M13 Forward and Reverse primers. ESTs were edited in silico to remove regions of low quality and sequences of vector, adapters and poli(A) tail using the softwares DNA Baser and SeqClean. Similarity searches were done using BLASTx and BLASTn programs. We obtained 358 sequences with good quality after edition. Transcripts derived from hosts, because of insect feeding, were considered as contaminants and were removed. A considerable variety of transcripts were isolated and they are consistent with their local of expression (anterior midgut); for instance, we observed transcripts coding for defensin, lysozyme and brasiliensin. The most abundant transcripts were similar to defensin, a conserved secreted protein from *Oncopeltus fasciatus* and a polyprotein from slow bee paralysis virus. 148 sequences did not match any sequences from the databases and are, possibly, *T. brasiliensis* specific genes. Sequences will be also clustered to produce contigs using the CAP3 assembler and submitted to analysis with InterProScan. **Supported by:**::FAPEMIG, CAPES, CNPq

VE007 - DISTRIBUTION OF TRIATOMINE FAUNA AND NATURAL INFECTION BY Trypanosoma cruzi IN THE SEMIARID OF THE STATE OF RIO GRANDE DO NORTE, BRAZIL

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The vector control campaigns directed to Triatoma infestans contributed for reducing the transmission of *Trypanosoma cruzi* in six Southern Cone countries. In order to understand the transmission dynamics of this parasite in the semiarid of the State of Rio Grande do Norte, Brazil, were conducted captures of triatomines in sylvatic, peridomestic and domestic ecotopes from different counties of central and western mesoregions. The insects were identified and intestinal contents examined by direct method, xenoculture and PCR. Of 559 captured triatomines the species were distributed at *Triatoma brasiliensis* (56.5%), *T. pseudomaculata* (22.2%), *Panstrongylus lutzi* (17.3%) and *Rhodnius nasutus* (4.0%). The species were captured in the larvae and adults, except *P. lutzi* exclusively adult. In the sylvatic ecotopes were captured *P. lutzi* (44%), *T. brasiliensis* (40%) and *T pseudomaculata* (16%). In the peridomestic environment were identified *T. brasiliensis* (66%), *T. pseudomaculata* (27%) and *R. nasutus* (7.0%), while at intradomicile was only found T. brasiliensis. The rate of infected triatomines in the wild environment, peridomestic and domestic were 50.6%, 13.2% and 58.3%, respectively. Natural infection of triatomines by *T. cruzi* was 28.9% and highest rate was observed in *P. lutzi* (75.2%) followed by *T. brasiliensis* (22.1%), *T. pseudomaculata* (14.4%) and *R. nasutus* (4.5%). These data showed high positivity of *P. lutzi* that allied to their ability to invade the domicile attracted by light and the growing process of destruction of native forest suggests the possibility of participating in the exchange between transmission cycles of the parasite. *T. brasiliensis* species was the only present in all ecotopes this reinforces its importance in relation to the ability to adapt at domestic environment, potential as a vector, and maintenance and exchange between the cycles of transmission in the semiarid, indicating the need for continuous epidemiological surveillance actions. **by:**:CNPq; CAPES; FAPERN

VE008 - Chickens complement system kills *Leishmania* infantum promastigotes and the vector saliva does not protect the parasites against it

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American visceral *Leishmania*sis (AVL) is caused by *Leishmania infantum* and transmitted by the bite of the *Lutzomyia longipalpis* female. The vector inoculates the parasite along with its saliva, which contains several immunomodulatory molecules, including complement system (CS) inhibitors. A recent study showed that *L. longipalpis* saliva can protect *L. infantum* promastigotes against death by human CS. In endemic areas, domestic chickens could play an important role maintaining the population of the vector, acting as an usual blood source. Despite this, chickens may also act in the zooprophylaxis of the disease as their blood kills *Leishmania* parasites through mechanisms not yet clarified. The aim of the present work was to investigate the importance of chicken´s CS in the parasite clearance and to check if the saliva of the vector could protect *L. infantum* promastigotes against avian CS. Firstly, flow citometry assays were done using promastigotes marked with propidium iodide (PI) incubated in the presence of different concentrations of fresh chicken sera at 37ºC. In all concentrations tested, sera were able to kill nearly 90% of the parasites. When the CS was inactivated by incubation at 56ºC for 30 minutes, the death was abrogated. A control was done with only *L. infantum* and PI incubated at 40ºC (chicken body temperature) and no death was observed. Thereafter, the same assays were done in the presence of *L. longipalpis* saliva. This treatment did not protect the parasites. This was truth even when the less concentrated sera (2%) was used. We may conclude that the complement system is a very important mechanism through which chickens kill *L. infantum* and that the saliva of the vector does not protect the parasite against chickens CS. **Supported by:**:FAPEMIG, CNPq

VE009 - Morphologic and cytogenetic approaches from hybrid crossover experimental between Triatoma lenti and T. sherlocki (Hemiptera: Reduviidae: Triatominae)

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Collections made in 1975 in Santo Inácio/BA, Cerqueira captured what he termed "wild triatomines." After experimental crosses with *Triatoma brasiliensis* and *T. lenti* was admitted that it is *T. brasiliensis* subspecies, obtaining fertile offspring. Papa in 2002 began the studies of these specimens and named it as *T. sherlocki*, a new species. This study aims to compare the morphological and cytogenetic hybrids from crosses between *T. lenti* and *T. sherlocki*. Morphological structures of male and female genitalia, and the eggshells were analyzed by scanning electron microscopy. Geometric morphometric analyses of wings were evaluated using the CLIC program. The cytogenetic aspects from stained testicles were performed by using the C-banding technique. The infertility of crosses between *T. lenti* females was 85% whereas with *T. sherlocki* female was less than 15%. All F1 hybrids showed large red spots on the connexive, red rings on the legs and reduced size of the wings, as well *T. sherlocki*. The geometric morphometry of wings presented an intermediate pattern in F1 and proximity to *T. sherlocki* in F2. Microscopy of the eggshells hybrids showed characteristics similar to native species. Through C-banding technique we observed that the F1 and F2 presented heterochromatic blocks in one or both chromosomes ends as the parental generation. The analysis of metaphases in F2 allowed to observe the fragmentation of chromosomes and therefore, monovalent and trivalent autosomes, a phenomenon named hybrid dysgenesis. Our dates suggests that *T. lenti* position in the complex *T. brasiliensis* remains under discussion and new approaches are necessary to better understanding the relationships among the species that compose this complex. **Supported by:**:CAPES

VE010 - Interaction of the hemipteran Oncopeltus fasciatus with the trypanosomatid Leptomonas wallacei: an insight into parasitism

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The hemipteran *Oncopeltus fasciatus* (milkweed bug) is naturally infected with the trypanosomatid *Leptomonas wallacei*. In the present study, we used adult male and female insects from both infected and *Leptomonas*-free colonies. Statistically significant differences were observed when the insects were comparatively analyzed with respect to weight, length, wing size, hemi-elytra size and fat body weight. Greater production of eggs in the ovaries and higher percentage of egg eclosion were observed in the colony of insects free of *L. wallacei*. Correspondingly, we found a higher number of follicles undergoing atresia in the ovaries of insects from the infected colony. Infected insects presented a delay in the development from first instar nymphs through adults, as compared to the uninfected ones. Similarly, infected females lived for a shorter period of time, as compared to the uninfected ones. The number of circulating hemocytes and the expression of the prophenoloxidase (PO) activating system were enhanced in infected adult insects. Likewise, using the thiobarbituric acid reactive substances (TBARS) assay we showed that guts and fat bodies from infected female insects showed higher amounts of malondialdehyde (MDA), which is an indicator of tissue damage by a series of chain reactions. A basic local alignment search tool (BLAST) was run against a local *O. fasciatus* transcriptome as database and sequences from the hemipteran *Acyrtosiphon pisum* as query. Essential genes related to development and immunity were found, such as juvenile hormones pathway enzymes, spaetzle, phenoloxidase and ecdysone-related molecules. Ongoing qPCR experiments show significant differences in the expression of these genes when infected and *Leptomonas*-free colonies were compared. Our data lead us to the conclusion that the infection of *O. fasciatus* by *L. wallacei* promotes a decrease in the fitness of these insects. Therefore, the interaction between these organisms fits into a parasitic pattern. **Supported by:**:Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular, FAPERJ, CNPq, CAPES,PIBIC-UFRJ

VE011 - Partial Purification and Characterization of Apyrase Activity from saliva of the Triatomine Bug Rhodnius prolixus

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The saliva of the haematophagous arthropod *Rhodnius prolixus*, a vector of Chagas disease, contains apyrase (EC 3.6.1.5) activity that facilitates blood-feeding by inhibiting the ADP-induced host platelet aggregation. Here we report the partial purification of proteins that probably represent the *R. prolixus* members of the apyrase family. Salivary glands content was injected into the Superdex 200 10/300 GL column and eluted with 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 5.0 mM CaCl2 buffer at a flow rate of 0.5 mL/min. The eluted peaks were monitored at 280 nm on fast protein liquid chromatography. The fractions containing activity were pooled and subjected to affinity chromatography (Hi-Trap Blue-Sepharose column). *In-gel* activities followed by silver staining suggest that proteins mediating salivary apyrase activity in *R. prolixus* may assembly into oligomers. Also, the partially purified *R. prolixus* apyrases are capable of cleaving nucleotide tri- and diphosphates in a calcium-dependent manner. The purification of these enzymes will allow detailed characterization of both their structure/activity relationships and role in inhibiting platelet aggregation during blood ingestion by these haematophagous insects. **Supported by:**:Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES

VE012 - Identification of triatomine immunogenic salivary antigens and evaluating their effectiveness as markers of contact for Rhodnius prolixus (Stal ,1859) (Hemiptera, Reduviidae): The role of salivary apyrase.

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Triatomines bugs are important haematophagous insects and vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease in America. Due to the direct contact between the saliva and the host immune system, the monitoring of the level of antibodies anti-saliva in persons from endemic areas would be a good alternative for entomological inquiries. Therefore, the present work aimed at the identification of immunogenic salivary antigens to evaluate their use as contact markers for triatomines. For such, we exposed groups of mice to low and high infestations of different triatomine species. After that, the most immunogenic antigens in mice's sera were identified by immunoenzimatic assays (ELISA) and Western Blot. The most immunogenic antigens were purified and evaluated for cross reaction among the species of triatomines by using ELISA. The results showed that the mice exposed to low and high infestations by R. prolixus and Triatoma infestans developed an anti-saliva specie-specific response. Responses to low and high infestations were not different in the serum of mice exposed to R. prolixus saliva. The western blot results showed that a protein with 48.4 kDa and another with 18.2 kDa were the most immunogenic molecules in R. prolixus saliva. The 48.5 kDa protein was purified and identified as a salivary apyrase. ELISA showed that the apyrase from R. prolixus can be recognized by antibodies produced in mice exposed to R. prolixus infestations, but it cannot be recognized by serum of mice exposed to T. infestans or Triatoma brasiliensis infestations. By searching the NCBI databases, we identified two types of apyrase expressed in R. prolixus salivary glands: one intracellular and another secreted (probably the antigen with 48.5 KDa founded in the Western blot). Our results indicate the salivary apyrase from R. prolixus as a promising contact marker for the triatomine infestations. **Supported by:**:CAPES

VE013 - Characterization of Humoral Immune Responses in Rhodnius prolixus

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Innate immunity in insects is the first defense line against several microorganisms. The humoral immune response, object of this research, consists basically on the production of antimicrobial peptides, a process controlled by three pathways: Toll, IMD and Jak/STAT. This study aims to contribute to the understanding of *Rhodnius prolixus* immune response against infection with bacteria, fungi and trypanosomatids. Fasting adult females were injected with Gram-negative or positive bacteria, sticked with zymosan or blood-fed. Other insects were infected with *Trypanosoma cruzi* and *Trypanosoma rangeli* by artificial feeding. Another set of insects was injected with double-stranded RNA for genes of the IMD pathway. After all these treatments, expression of several putative immune-related genes was analyzed by qPCR. In insects silenced for Relish and Caspar, key genes from the IMD pathway, we additionally analyzed intestinal microbiota. The obtained results indicate that the Toll pathway is activated in the fat body in response to fungal infections and in the midgut after a blood meal. 24 hours after feeding, IMD pathway is also activated in both tissues. IMD pathway was also activated in the fat body in response to Gram-negative bacteria. Interestingly, infection with both *Trypanosoma cruzi* or *Trypanosoma rangeli* inhibited the expression of several immune genes, suggesting that the parasite might induce a state of “immunosuppression” in the vector in first days of infection. Silencing of genes from the IMD pathway showed that defensin seems to be under control of this pathway, while lysozyme-A, lysozyme-B and cecropin-2 do not. The knockdown of Relish, the transcription factor of the IMD pathway, leads to a major increase in the amount of bacteria in the gut, implicating this pathway in control of intestinal microbiota. Additional studies are still needed to confirm these data. **Supported by:**:CNPq / FAPERJ

VE014 - Fitness of kdr mutation in the dengue vector mosquito Aedes aegypti

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Pyrethroids insecticides target the voltage gated sodium channel (Na_V), inducing the knockdown effect. In *Aedes aegypti*, the main dengue vector, the $AaNa_V$ substitution Val1016Ile is known as the knockdown resistance (kdr) mutation. We evaluated the fitness cost of this mutation upon distinct aspects of development and reproduction, in the absence of any other major resistance mechanism. Initially we settled up crosses using 68 couples from a natural population. Allele specific PCR revealed that only one couple, the one originating the strain CIT-32, had both parents homozygous for the mutant allele. However, this strain also presented high levels of detoxifying enzymes, which synergistically account for resistance, as revealed by biological and biochemical assays. Therefore we backcrossed CIT-32 with Rockefeller, a laboratory reference strain, for eight generations in order to bring the kdr mutation into a susceptible genetic background. This new strain, named Rock-kdr, was highly resistant to pyrethroid and presented reduced alteration of detoxifying activity. Fitness of Rock-kdr was then evaluated in comparison with Rockefeller. In the selected strain, larval development took longer, adults had an increased locomotor activity, fewer females laid eggs and in a smaller number. By contrast, adult body weight, longevity, amount of ingested blood and rate of egg eclosion were similar to Rockefeller. Under an inter-strain competition scenario, Rock-kdr larvae developed even slower. Moreover, when Rockefeller and Rock-kdr were reared together in population cage experiments during 15 generations in absence of insecticide, the mutant allele decreased in frequency. These results strongly suggest that the *Ae. aegypti* kdr mutation Val1016Ile has a high fitness cost. Therefore the use of pyrethroids should be suspended in localities where the kdr mutation is found before new adaptive alleles can be selected for diminishing the kdr deleterious effects. **Supported by:**:CAPES, CNPq-Pronex Dengue, INCT-Entomologia Molecular

VE015 - Epidemiological Evaluation of Phlebotomine (Diptera: Psychodidae) Fauna in Area of Coverage in The Field of Instituto Evandro Chagas, Municipality of Ananindeua-Pa, Brazil

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*Leishmania*sis is a widely distribution disease been taken place in 88 countries. The disease is caused by an obligated intracellular protozoan of genus *Leishmania* which is transmitted by the bite of phlebotomines sand flies during blooding sucking in vertebrate host. There are three clinical forms of the disease, cutaneous, mucocutaneous and visceral *Leishmania*sis. In Brazil, some phlebotomine species as *Lutzomyia (Nyssomyia) whitmani* and *Lutzomyia (Nyssomyia) flaviscutellata* are related in transmitting cutaneous and mucocutaneous forms respectively and *Lutzomyia (Lutzomyia) longipalpis* is the main vector of *Leishmania (Leishmania) infantum chagasi* the etiological agent of visceral *Leishmania*sis. The aim of this study was to identify the phlebotmine fauna in a pocket of residual primary forest surrounded by urban areas. The study area belongs to Instituto Evandro Chagas (IEC) in municipality of Ananindeua-PA, Brazil. Systematic captures of phlebotomines were performed using CDC light traps installed in canopy and ground (1,5 m) and Shannon traps. The captures were carried out in the field II of IEC from October 2010 to October 2011. A total of 2,675 specimens belonging to twenty-four species and two genus were captured. The most abundant species was *Lutzomyia (Nyssomyia) antutesi* (50,09%) most of them being females (70%). Other two species *L. flaviscutellata* (1,4%) and *L. longipalpis* (0,4%) have drawn the attention for being vectors of *Leishmania (L.) amazonensis* and *Leishmania (L.) chagasi* respectively. The females were dissected for microscope analysis. One specimen of *Lutzomyia rorotaensis* was positive for flagellates. The presence of vectors of *Leishmania (Viannia) lindenbergi, L. chagasi* and *L. (Leishmania) amazonensis* captured in the area showed the necessity of entomological monitoring in municipality of Ananindeua-Pa.

VE016 - Evaluation of the voltage gated sodium channel gene diversity of natural Anopheles populations from the Amazon region and its relationship to pyrethroid resistance

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The main tool against malaria vectors still relies on the use of pyrethroid insecticides, applied in spatial sprayings or impregnated in nets or curtains. Except for the use of DDT in some African localities, pyrethroids are the most used insecticides due to their immediate action, ease of application and reduced environmental impact. However the massive use of these compounds results in selection of resistant anopheline populations worldwide. The target site of both DDT and pyrethroids is the voltage gated sodium channel (Na_V) of insect nerve cells, leading to convulsions and paralysis followed by death (the *knockdown* effect). Resistant individuals are known as *kdr* (*knockdown* resistant). The main molecular mechanism for this trait is a conserved mutation on the Na_V 1014 aminoacid, placed in the IIS6 segment of the channel. The *kdr* Leu1014Phe mutation has been found in a series of insect species, including several anophelines from Africa and Asia. In Brazil, albeit reports of resistance in natural Amazonian Anopheles populations, their Na_V molecular diversity is still unknown. Here we present the genomic diversity of the Na_V IIS6 region of *Anopheles darlingi* and *Anopheles marajoara* natural populations from localities of Amapá State/ Brazil. We also designed primers for allele-specific PCR (AS-PCR) for large scale individual genotyping of the 1014 site (alleles Leu or Phe). However, the *kdr* allele has not yet been found in any genotyped individual (from S Navio, Mazagão, P Grande and Oiapoque). These results, although preliminary, indicate that this common pyrethroid resistance mechanism is absent (or at least under low frequency) from the main malaria vector populations from Amapá State. Therefore the use of pyrethroid treated materials and house spraying as well might still be an efficient complementary strategy on those localities, whenever the susceptibility status of vector populations is continually monitored. **Supported by:**:Conselho Nacional de Desenvolvimento Científico e Tecnológico

VE017 - Bioluminescence Imaging of *Trypanosoma cruzi* Infection in Rhodnius prolixus

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Usually the analysis of the various developmental stages of *Trypanosoma cruzi* in the experimentally infected vertebrate and invertebrate hosts is based on the morphological observations of tissue fragments. The development of techniques that allow the imaging of animals infected with parasites expressing luciferase open up possibilities to follow the fate of bioluminescent parasites in infected vectors. The luciferase gene was integrated into the genome of the Dm28c clone of *T. cruzi*, and used to follow, in real time, the infection of the insect vector *Rhodnius prolixus*, by a non invasive method. The insects were evaluated by in vivo bioluminescence imaging on the feeding day, and on the 7 th, 14 th, 21 st and 28 th days after feeding. D-luciferin (60 µg) was injected into the hemocel of the whole insect before bioluminescence acquisition in the IVIS® Imaging System, from Xenogen. To corroborate the bioluminescence imaging made *in vivo* and distinguish the digestive tract region that was infected, the insects were dissected, and the whole gut incubated with D-luciferin for *ex vivo* evaluation in the IVIS® Imaging System. The same digestive tracts were also macerated for parasite counting of morphologically distinct stages, in the optical microscope, and for bioluminescence acquisition in the microplate by the IVIS® Imaging System. A positive correlation of parasite number and bioluminescence emission in the microplate was obtained. This is the first report of bioluminescence imaging in *Rhodnius prolixus* infected with trypomastigotes of Dm28c-luc stable strain, expressing firefly luciferase. In spite of substrate (D-luciferin) distribution limitations in the insect body for longitudinal evaluation of infected insects, bioluminescence imaging of digestive tract infected with Dm28c-luc is highly sensitive and accurate to track the fate of the parasite in the vector, in the crop, intestine and rectum. **Supported by:**:FAPERJ, CNPq and CAPES

VE018 - Kdr mutation in Culex quinquefasciatus natural populations from Brazil

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Pyrethroid insecticides target the voltage gated sodium channel (Na_V) of insect nervous cells, triggering repetitive convulsions followed by paralysis and death (*knockdown* effect). Their intense use selects resistance in various species of agricultural and health importance. A punctual mutation in the Na_V gene is related to pyrethroid resistance in insects of several orders (Leu1014Phe), being commonly referred to as *kdr* mutation (*knockdown* resistance). In Brazil, *Culex quinquefasciatus* is the vector of lymphatic filariasis in some regions and despite awareness of resistance to pyrethroids, the molecular Na_V gene diversity in this species ($CqNa_V$) has never been explored. Evaluation of the $CqNa_V$ molecular diversity from Brazilian populations is here presented, in the search for association with pyrethroid resistance. Adult *Culex* mosquitoes collected by Health Secretariat from several States were kindly sent to our laboratory. For a first panorama, we cloned and sequenced the genomic region spanning the *kdr* mutation from pools of natural populations from each locality. Following, we developed primers for allele specific PCR able to discriminate wild type (1014 Leu) and mutant (1014 Phe) alleles. By this technique we genotyped 453 individuals, distributed in 12 populations: Oiapoque, Perpétuo do Socorro and Capivara (AP); Natal (RN); Campo Grande (MS); Duque de Caxias, Autódromo, Jacarepaguá, Colônia, Cabuçu, Moqueta, Cerâmica and Vilas da Barra (RJ). The *kdr* mutation Leu1014Phe was identified in only two populations, Autódromo and Campo Grande. Another mutation in the same site (Leu1014Ser) is being investigated, through Restriction Fragment Length Polymorphism-like assay. Not only are these tools important to access the *kdr* frequency in natural populations but also to predict their actual profile of resistance to pyrethroids, the most used class of insecticides. **Supported by:**:Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro

VE019 - Reproductive biology alterations in Rhodnius prolixus infected by *Trypanosoma rangeli*

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The parasites can change the behavior of the host as a way to increase your chances of completing its life cycle. These changes can affect the reproductive mechanisms of the insect vector. Although the reproductive behavior of some triatomine species has previously been studied, the impact of parasitic infection on the reproductive biology has not yet been fully evaluated. In order to contribute to the basic knowledge of reproductive biology of insects and identify reproductive patterns caused by an infection in the insect vector. In this work, we are studying the reproductive biology associated with the pathogenic interaction between T. rangeli and R. prolixus. Adult virgin females of R. prolixus were fed on blood meals containing T. rangeli epimastigotes. Blood meals were prepared by centrifugation of citrated sheep blood. Erythrocytes were added to plasma after inactivation by heat to complete the original blood volume. In addition, T. rangeli epimastigotes, Macias strain, was added at 1x106 flagellates /ml of blood for the oral infection. Control insects were fed on blood without parasites. All of females groups were placed to mate with virgin adult male control and the couples were formed. In order to maintain females' homeostasis, these were fed weekly on blood from Swiss mice (CEUA Protocol - LW-14/10 FIOCRUZ). Our results demonstrated the presence of T. rangeli in the hemolymph of infected females until 40 days after infection. In addition, the infected females' oviposition was greatly altered when compared to the control group, 5 eggs and 42 eggs respectively for 40 days. In this context, our results demonstrate that the presence of the parasite may influence the number of eggs laid by the females. Additional experiments are underway to clarify the physiological effects of the impact of parasitism on reproduction and egg laying of R. prolixus. **Supported by:**:Faperj

VE020 - Duox activity in ovaries of Rhodnius prolixus is essential for eggshell waterproofing

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The insect eggshell, or chorion, is a multilayered structure that confers physical and biological protection during embryo development. In Drosophila melanogaster and Aedes aegypti, it was shown that the chorion proteins are insolubilized by covalent cross-linking via dityrosine bonding, a process that confers the egg resistance against water loss. This process is catalyzed by chorion peroxidases, at the expense of H2O2. The family of NADPH oxidases includes the dual oxidase (duox) enzymes that generate H2O2, and show an additional peroxidase domain when compared to typical NADPH oxidases. In the present work we have identified a Duox gene in Rhodnius prolixus that is expressed in ovaries of adult females, especially in the follicular epithelia during choriogenesis. Inhibition, by means of RNAi, of Duox expression leads to a decreased H2O2 generation in the ovary and the production of eggs showing lower levels of dityrosine bonds in the chorion. These eggs showed a disturbance in the egg waterproofing process and dried after oviposition. In this way, Duox-RNAi caused a marked decrease of the eclosion ratios, which was reverted when the eggs were incubated at > 96% relative humidity, when Duox-silenced females had simultaneously the catalase expression inhibited, or when H2O2 was administered via injection into their hemocoeles. Thus, we suggest that H2O2 generated by Duox is essential for the peroxidative crosslinking of chorion proteins and consequently for the egg waterproofing in R. prolixus. **Supported by:**:Capes/Faperj